



TechNote #TNGK200.1

COMPARING PNGASE F ACTIVITY

Genzyme's patent for recombinant PNGase F in Europe (EP Patent No. 0472651 B1, Endo F-Free PNGase F) was upheld May 4, 2006. Users, who wish to purchase from a supplier licensed under the patent, have inquired about the conversion of units from Roche Applied Science (Roche) to ProZyme's N-Glycanase®.

A review of technical support documents available on-line reveals that the major differences between N-Glycanase and recombinant PNGase supplied by Roche are:

- Unit Definition - ProZyme defines 1 Unit as the amount of enzyme which catalyzes the release of N-linked oligosaccharides from **1 micromole** of denatured ribonuclease B (RNase B) per minute at pH 7.5 and 37°C. Roche defines 1 Unit as the enzyme activity which hydrolyzes **1 nanomole** of dabsyl fibrin glycopeptide per minute at pH 7.8 and 37°C. Based upon these definitions, one Roche unit is equivalent to one ProZyme milliunit.
- Assay Substrate - ProZyme uses denatured ribonuclease B while Roche specifies dabsyl fibrin glycopeptide. Both of these substrates have been standardized against native PNGase F from *Flavobacterium meningosepticum* according to the procedures defined by Plummer and Tarentino (1984, 1985 and 1987).

Differences in substrate specificity could, in principle, lead to differences in the measured specific activities of the ProZyme and Roche enzymes. However, in practice, we find that the measured activity of the Roche enzyme using RNase B as the substrate is the same as the activity reported by Roche for the enzyme, indicating that both substrates

Table 1 - N-Glycanase® vs. Roche PNGase F

Product Code (Cat. No.)	nominal U/ml (Roche)	equivalent U/ml (ProZyme)	Qty ¹ (µl)
GKE-5006	n/a	2.5 U/ml	4
GKE-5010	n/a	10 U/ml	1
11 365 169 001 11 365 177 001	1,000 U/ml	1 U/ml	10
11 365 185 001 11 365 193 001	1,000 U/ml ²	1 U/ml	10

¹ Volumes recommended to obtain comparable digestion, e.g. 1 µl of ProZyme GKE-5010 is equivalent to 10 µl of Roche 11 365 169.

² when dissolved as directed

give substantially the same apparent activity. Therefore, we conclude that Roche PNGase F has the same specific activity as ProZyme N-Glycanase after correcting for the different unit definitions.

- Concentration - ProZyme offers N-Glycanase in two concentrations, 2.5 U/ml and 10 U/ml, while Roche offers a liquid formulation at 1 U/ml (1,000 U/ml Roche Units) and a lyophilized version with instructions to dissolve to a concentration of 1 U/ml (1,000 Roche Units). Table 1 shows suggested equivalent volumes when substituting ProZyme's N-Glycanase for Roche PNGase F.

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- Formulation - ProZyme N-Glycanase is formulated in 20 mM Tris-HCl and 50 nM EDTA (pH 7.5). The Roche liquid formulation is 50 mM sodium phosphate, 12.5 mM EDTA and 50% glycerol (v/v), pH 7.2; the lyophilizate, when dissolved as directed, results in a formulation of 100 mM sodium phosphate buffer and 25 mM EDTA (pH 7.2). These differences should not affect enzyme activity.

METHODS

Two samples of Roche PNGase F were obtained:

- Cat. No. 11 365 177 001, lot 10610423, nominally 250 Units in 0.25 ml, reported to be 25,000 U/mg specific activity and 1,000 U/ml activity. Calculated protein concentration is 0.04 mg/ml.
- Cat. No. 11 365 193 001, lot 12576121, nominally 250 Units lyophilized. When dissolved as directed, reported to be 1,000 U/ml of activity.

Protein concentration (BCA) and enzyme activity were performed using ProZyme's standard protocols. Results confirmed the protein concentration and activity as expected. If both enzymes behave the same relative to other glycoproteins as in the ProZyme activity assay, it should be possible to substitute an equal mass of ProZyme N-Glycanase for Roche PNGase F in any

assay to obtain the same results (~1 Unit of N-Glycanase for each 1000 Roche Units). Table 1 shows equivalent activity, concentrations and suggested volumes to use to achieve equivalent deglycosylation.

REFERENCES

- Plummer, T. H. Jr, J. H. Elder, S. Alexander, A. W. Phelan and A. L. Tarentino. Demonstration of peptide:N-glycosidase F activity in endo- β -N-acetylglucosaminidase F preparations. **J Biol Chem** **259(17)**: 10700-4 (1984).
- Tarentino A. L., C. M. Gomez and T. H. Plummer Jr. Deglycosylation of asparagine-linked glycans by peptide:N-glycosidase F. **Biochemistry** **24(17)**: 4665-71 (1985).
- Tarentino A. L. and T. H. Plummer Jr. Peptide-N₄-(N-acetyl- β -glucosaminyd) asparagine amidase and endo- β -N-acetylglucosaminidase from *Flavobacterium meningosepticum*. **Methods Enzymol** **138**: 770-8 (1987).

TECHNICAL SERVICE

This and other TechNotes are available on ProZyme's webpage located at:

<http://www.prozyme.com/technical/index.html#technotes>

ProZyme customers are an important source of information regarding advanced or specialized uses of our products. We encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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